CHRISTOPHER

The Separation of Invertase Catalase and Peroxidase by Selective Extraction from Yeast

Zoology

B. A.

1908

THE SEPARATION OF INVERTASE CATALASE AND PEROXIDASE BY SELECTIVE EXTRACTION FROM YEAST

BY

DANIEL LEROY CHRISTOPHER

THESIS FOR THE DEGREE OF BACHELOR OF ARTS
IN ZOOLOGY

COLLEGE OF SCIENCE
UNIVERSITY OF ILLINOIS

PRESENTED, JUNE, 1908

UNIVERSITY OF ILLINOIS

May 30, 1908.

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Daniel Leroy Christophes

ENTITLED The Separation Of Invertase Catalase And Peroxidase By Selective Extraction From Yeast.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor Of Arts in Zoology.

A. M. Peters
Instructor in Charge.

APPROVED: S. A. Forbes

HEAD OF DEPARTMENT OF Zoology

The Separation Of Catalase, Peroxidase And Invertase By Selective Extraction From Yeast.

Table Of Contents.

3.2 Catalase.

Method of extraction. Time of extraction and repeated extractions. Amount of enzyme. Tests. Separation by successive extraction, with different media. Blank and control experiments. Critical review on catalase.

3.3 Peroxidase.

Method of extraction. Time of extraction and repeated extractions. Amount of enzyme. Tests. Separation by successive extraction, with different media. Blank tests and controls. Critical review on peroxidase.

4. Critical review and summary of results.

1. Introduction.

The investigation described in this paper was begun in February 1908 and is now not quite finished. It has however proceeded far enough to develop data of significance in the field in which it deals.

The purpose of the work was to test the power and selective action of several liquid media for the extraction of one or more of the numerous enzymes contained in the yeast cell. The motive for the selection of the particular media here tested came from other investigations of this laboratory. It was hoped that on account of the different permeabilities of the cells towards these media or on account of the different solubilities of the ferments a selective extraction and consequent separation of the ferments might be discovered. By combining selective extraction with appropriate after treatment of the extracts thus obtained the end sought has been practically accomplished. The experiments have been confined to the enzymes catalase, peroxidase and invertase.

2. Origin And Preparation Of Raw Materials And Of Extracting Agents.

At first ordinary commercial yeast, such as Yeastfoam, was used. It was soon found that owing to the large amounts of impurities in this yeast the results could not be relied upon. This yeast, so called, was much lacking in yeast cells, being made up mostly of nutrient matter for the development of the few yeast cells that were present.

Fleischmann's compressed yeast (a damp, pressed yeast) was adopted for this work. An important factor in favor of this yeast was its richness in yeast cells. It was found that, by washing with distilled water, practically all of the extraneous matter except part of the starch, was removed. Since it was known that starch had no effect upon the enzymes in question, it was not deemed necessary to try to remove it completely.

The washing was accomplished as follows: The yeast cake was broken into bits, placed in a tall vessel and covered with five or six volumes of distilled water. This mixture was well shaken and allowed to settle. After about five minutes the water was removed by a siphon. The solid matter consisting of yeast cells and of a little starch was found to be very clean after four or five washings.

A little yeast was lost by this method, but, compared with the amount of foreign matter removed, the loss was insignificant.

Liver.

The liver was such as is obtained in the market suitable for food. After washing with distilled water the necessary amount was weighed out, cut into little bits and placed under extracting agents.

Reagents.

The following reagents were used in the indicated concentrations:

1. Thymol water-0.5 saturated solution or 0.15%.
2. Sodium fluoride (NaF)-2% solution.
3. Sodium hydroxide (NaOH)-0.01 normal solution.
4. Acetic Acid (CH_3COOH)-0.015 normal solution.
5. Zinc sulphate ($ZnSO_4$)-0.05 saturated solution or about 6.7%.

The thymol was used in the water, merely as a disinfectant and an antiseptic. The thymol water was made as follows:

3 Gm. of thymol were dissolved in 1000 cc. of hot distilled water. Upon cooling some of the thymol crystallized out, leaving a saturated solution.

The reagents were made up double the above indicated concentrations. To use them, one volume of the reagent was mixed with an equal volume of yeast suspended in water. In all cases about 10 Gm. of solid material were allowed to each 100 cc. of the reagent.

5. The Extraction of Enzymes
Experiment Number 8-4-10/1.

On Invertase.

In order to determine whether or not invertase could be extracted from yeast and to determine which of the reagents were the most efficient, the following flasks were made up and allowed to stand at room temperature about 20°C. for 72 hours.

A-yeast in Thymol water- 0.5 saturated.

B- " " NaOH - 0.01 normal.

C- " " CH_3COOH - 0.015 normal.

D- " " NaF - 2%.

E- " " $ZnSO_4$ - 0.05 saturated i.e. 6.7% about.

When the flasks had stood for the required length of time, 2 cc. of the liquid was removed from each and placed in flasks containing cane sugar. These flasks were disgnated and made up as follows;-

Extracts.

a1 -40 cc. of 10% cane sugar solution 2 cc. extract from A

b1 - " " " " " " " " " " " " " B

c1 - " " " " " " " " " " " " " C

d1 - " " " " " " " " " " " " " D

e1 - " " " " " " " " " " " " " E

In order to have a control on each of the above tests and to find the amount of inversion if any due to the reagents the following flasks were made up:-

a2-40 cc. of 10% cane sugar solution 2cc. glycol water.

b2- " " " " " " " " " NaOH.

c2 -" " " " " " " " " CH_3COCH.

d2- " " " " " " " " " NaF.

e2- " " " " " " " " " $ZnSO_4$.

The flasks were incubated 24 hours at 40°C, after which, the amount of invert sugar was determined by Schoorl's method.

Schoorl's method of estimating sugar is a modification of Fehling's and is essentially as follows:-

12 Cc. of the sugar solution was placed in a Jena glass flask with 8 cc. of Fehling's solution. This mixture was boiled for exactly two minutes and then cooled under the tap as quickly as possible. After cooling 7 cc. of a 10% K I solution and 4 cc. of 15% H_2SO_4 were added in order mentioned. A few drops of a 1% starch solution were added. This starch became blue from the liberated I. The amount of free I was determined by titrating with a 0.1 normal solution of $Na_2S_2O_3$ until the color disappeared. The blank value was determined by boiling 12 cc. of distilled water with 8 cc. of Fehling's solution for two minutes, and proceeding as described above. The difference between the number of cc. of $Na_2S_2O_3$ required to remove the color from the blank, and the number of cc. required to remove the color from the test gives the $Na_2S_2O_3$ difference. From the $Na_2S_2O_3$ difference the amount of sugar may be read off or interpolated in the appended table given by Schoorl. If the solution contains so much sugar that all of the copper is reduced upon boiling,-i.e. all of the blue color disappears from Fehling's solution-it must be divided, but the volume of the liquid tested must always remain the same,i.e. 12 cc.

Table 1

Schoorl, N. '99 Zur Jodometrischem Zuckerbestimmang mittels Fehlingscher Lösung Zeitschr. f. angew. Chem. 1899 pp. 633-635.

cc. 1/10 n. Thio	$C_6H_{12}O_6$. Glucose mg.	$C_{12}H_{22}O_{11}$ Saccharose mg.	$C_6H_{10}O_5$ Amylum mg.	$C_{12}H_{22}O_{11}H_2O$ Lactose mg.
1	3.2	3.1	2.8	4.5
2	6.3	6.2	5.7	9.0
3	9.4	9.3	8.5	13.6
4	12.6	12.4	11.4	18.1
5	15.9	15.6	14.3	22.7
6	19.2	18.8	12.3	27.3
7	22.4	22.2	20.2	31.9
8	25.6	25.2	23.1	36.5
9	28.8	28.4	26.1	41.1
10	32.3	31.7	29.1	45.8
11	35.7	35.0	32.1	50.6
12	39.0"	38.3	35.1	55.4
13	42.4	41.6	38.2	60.3
14	45.8	44.9	41.3	65.2
15	49.3	48.2	44.4	70.1
16	52.8	51.6	47.5	75.0
17	56.3	55.1	50.7	80.0
18	59.8	58.7	53.9	85.0
19	63.3	62.3	57.1	90.0
20	66.9	65.9	60.3	95.0
21	70.7	69.9	63.7	100.0
22	74.5	73.3	67.1	105.0
23	78.5	77.1	70.1	110.4

cc.	mg.	mg.	mg.	mg.
24	82.6	80.9	74.3	115.9
25	86.6	84.7	77.9	121.6
26	90.7	88.6	81.6	127.5
27	94.8	92.5	85.3	133.8

The results of this series of experiments are shown in Table II.

Table II.

REAGENTS

1	Blank	Thymol water a2	NaOH b2	CH_3COOH c2	Naf d2	$ZnSO_4$ e2
2 No. cc. of solution taken		12	12	12	12	12
3 No. cc. of $Na_2S_2O_3$	10.55	8.85	8.75	2.35	8.05	9.45
4 $Na_2S_2O_3$ difference for 12 cc. of solution		1.70	1.80	8.20	2.50	1.10

EXTRACTS

5	Thymol water a1	NaOH b1	CH_3COOH c1	NaF d1	$ZnSO_4$ e1
6 No. cc. of solution taken	1	1	1	1	1
7 No. cc. of $Na_2S_2O_3$	9.05	9.45	7.95	9.35	10.35
8 $Na_2S_2O_3$ difference for 1 cc. of solution	1.50	1.10	2.60	1.20	0.20
9 No. cc. of $Na_2S_2O_3$ equal to 12cc. of sugar solution (=line 8 x 12)	18.00	13.20	31.20	14.40	2.40

	Thymolwater	NaOH	CH_3 COOH	NaF	Zn SO_4
10 No cc. of Na_2 S_2 O_3 equal to 12 cc. of sugar solution, Invert sugar caused by Invertase. (= Line 9 − line 4)	16.30	11.40	23.00	11.90	1.30

Line 2 shows the amount of the solution used in the test. The amount of invert sugar was so large in the case of the extracts that 1 cc. of the solution was diluted to 12 cc. with distilled water, and used as the method required. Line 3 shows the actual number of cc. of Na_2 S_2 O_3 used to remove the color from the solution. Line 4 shows the number of cc. of Na_2S_2 O_3 difference between the blank and the number used in titrating 12 cc. of the reagent, while line 8 shows the Na_2 S_2 O_3difference for 1 cc. of the sugar solution inverted by the yeast extracts. Line 9 shows the number of cc. of Na_2 S_2 O_3required to reduce the amount of Fehling's solution that 12cc. of the solution will reduce. Line 10 shows the same as 9 except that the amount indicated there is the reducing power of invert sugar caused by invertase alone.

The above table shows that invertase can be gotten into solution, in rather large amounts, quite easily. For this purpose acetic acid is much the best, although thymol-water and other reagents can be used to great advantage.

The main fault with the data of the above table is that they do not show the action of Zn SO_4 upon invertase, for from the data there shown two things are possible. Either invertase was present but its action hindered by Zn SO_4, or it was not

extracted from the yeast at all.

Experiment 8-4-23/1 On the Action Of Zn SO_4 Upon Invertase. In order to show the action of Zn SO_4 (0.05 saturated solution) upon invertase in solution, the following tests were made:-

In a flask markedA, was placed 40 cc. of a 10% solution of cane sugar and 2 cc. of a NaF extract of yeast, and allowed to stand.

In the flask B, was placed the same amount of a 10% cane sugar solution and 2 cc. of a NaF extract of yeast, which extract was mixed with Zn SO_4 solution as follows 1 cc. of a 0.5 saturated solution Zn SO_4 was diluted to 10 cc. by adding some of the NaF extract used in A, thus leaving the solution of Zn SO_4, 0.05 saturated. After standing the required length of time the amount of invert sugar was determined by Bang's method.

Bang's Method is essentially as follows:- A copper solution was made by dissolving 250 gm. of potassium carbonate, 200. gm. of potassium thiocyanate, and 50. gm. of primary potassium carbonate in about 600. cc. of water and cooled. After cooling, this solution was mixed with a solution consisting of 12.5 gm. of purified copper sulphate pentahydrate in 75.cc. of water. This mixture was allowed to stand 24 hours, then filtered and made up to 1000.cc.

A hydroxylamine solution was made by dissolving 6.55 gm. of the sulphate and 200. gm. of potassium thiocycnate making up to 2000.cc.

In practice 5 cc. of the copper solution was run into a

large test tube and 1 cc. of the sugar solution was added. This was then boiled for three minutes cooled under the tap and titrated to colorless by the hydroxylamine solution. The blank test was made by titrating to colorless 5 cc. of the copper solution.

The difference between the hydroxylamine value of the blank and the tests show the relative amounts of invert sugar.

The results from these tests are shown in table III.

Table III

Temp	hrs.	Blank	A	B	Bl.-A	Bl-B	dil	
20°c	4	3.4	2.4	2.4	1.0	1.0	10 volume	1
40°c	24	3.4	1.2	1.3	2.2	2.1	20 volumes	2

The colums marked A and B show the no. of cc. of hydroxylamine necessary to remove the color from the copper solution after being boiled with the inverted sugar solution. The columns marked Bl-A and Bl-B show the number of difference between the blank and solutions A and B. The column marked dil. shows the amount of dilution necessary on account of the large amount of invert sugar present. 1 cc. of the solution in line 1 was diluted to 10 volumes and 1 cc. of the dilute solution was tested.

From the above table it is evident that Zn SO_4 does not inhibit the action of invertase in solution, for if investase is present in aZn SO_4 solution its inverting power

remains and its character as an enzyme is not changed.

From the data shown in tables II and III it is evident that Zn SO_4 will not extract invertase from yeast.

Experiment 8-4-28/2 on Invertase.

Having found that Zn SO_4 will not extract invertase from yeast the next step was to follow this extraction with NaF. Accordingly the following tests were set:-

A- Yeast which had been under three changes of Zn SO_4. The first was removed after 120 hours, the second after 72 additional hours, and the third after 48 additional hours. After the third extract had been removed NaF was placed on the yeast for 24 hours, at the end of that time 2 cc. was removed and placed in 40 cc. of 10% cane sugar solution.

B- Yeast which had been under three changes of NaF as that in A, was treated in a similar manner.

The relative amounts of invert sugar at different periods and temperatures were estimated by Bang's method, as shown in table IV.

Table IV.

	Temp.	hrs.	blank	A	B	B1-A	B1-B	dil.
1	20°c	4	3.4	3.4	3.35	--	0.05	none
2	40°c	24	3.4	3.15	3.35	0.20	0.05	none

This table is exactly like table III. From the above table it is seen that invertase is left unchanged in the yeast by the Zn SO_4 extracting agents, even after repeated extractions. Furthermore if the extraction by Zn SO_4 if followed by NaF the invertase can be gotten in the solution.

Experiment 3-4-28/1 On Adsorption Invertase.

The following series of experiments were instituted in order to prove whether or not invertase could be removed or rather adsorbed from a solution by a precipitate.

For this purpose a 10% suspension of purchased dehydrated $Zn_3 (P O_4)_2$ was made. To 10 cc. of the extract of yeast 2.5 cc. of the suspension was added and after having been thoroughly mixed together, the $Zn_3 (PO_4)_2$ was allowed to settle. Both the $Zn_3 (PO_4)_2$ e.g. A1, B1, C1, and the liquid above e.g. A3 B3 C3 were tested for invertase.

The Reagenta were tested in a manner similar to the extrat in order that the absolute value as nearly as possible of the invertase could be found, therefore A_2, B_2, C_2 and A 4, B4, C4 respectively composed of reagents bear the same relation to each other as A 1 to A 3 etc among the extracts.

The flasks were made up as follows:

Extract = A1 = $Zn_3 (PO_4)_2$ from NaOH Extract + 40cc sugar sol.
Reagent = A_2 = " " NaOH Reagent + " " "
Extract = A_3 = liquid " NaOH Extract + " " "
Reagent = A_4 = liquied " NaOh Reagent + " " "
Extract = B_1 = $Zn_3 (PO_4)_2$ " Na F Extract + " " "
Reagent = B_2 = " " " Reagent + " " "
Extract = B_3 = liquid " " Extract + " " "
Reagent = B_4 " " " Reagent + " " "
Extract = $Cl = Zn_3 (PO_4) 2 Zn SO_4$ " " Extract + " " "
Reagent = C_2 " " " " Reagent + " " "
Extract = C_3 = liquid " " Extract + " " "
Reagent C_4 = " " Reagent + " " "

Table V

			Na OH				NaF				$ZnSO_4$			
			Ext.	Reag	Ext.	Reag.	Ext.	Reag	Ext.	Reag	Ext.	Reag	Ext.	Reag
1.	hrs.	Blank	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
2	3	3.65	3.45	3.65	3.55	3.60	2.75	3.55	3.10	3.55	3.30	3.60	3.60	3.65
3	24	3.70	2.80	3.70	3.40	3.70	1.55	3.45	0.15	3.50	3.40	3.60	3.65	3.65
4	3		0.20		0.05		0.80		0.45		0.30		0.05	
5	24		0.90		0.30		2.00		3.35		0.20		—	

In table V lines 2 and 3 show the number of cc. of hydroxylamine used in titrating. Line 4 and 5 show the number of cc. of hydroxylamine it would have taken if invertase alone had acted upon the cane sugar.

From this it is seen that where the amount of invertase is small the greater part of it is adsorbed but where the amount is large much of it is left in solution, indicating that the capacity of $Zn_3(PO_4)_2$ for holding invertase in adsorption is limited i. e. given quantity of the adsorbent has certain saturation limits.

Experiment 8-4-30/1 On Adsorption of Invertase

Thinking that the purchased $Zn_3(PO_4)_2$ was too dry and granular it was decided to make some in the laboratory. Equal volumes of a 0.25 molecular solution of $Zn\ SO_4$ and of a 1.0 molecular solution of $KH_2\ PO_4$ were mixed together, thoroughly shaken and centrifuged. The liquid was poured off from above the precipitate. The precipitate was washed several times with distilled water and then used. The precipitate from 2 cc. each $Zn\ SO_4$ and $K\ H_2\ PO_4$ was used to each 10 cc. of the extract.

Extract = B1 = $Zn_3 (PO)_4 2$ from NaF extract + 40 cc. sugar sol.

Reagent = B2 " " " reagent + " " "

Extract = B3= liquid " " extract + " " "

Reagent = B_4 " " " reagent + " " "

Extract = C1= $Zn_3 (PO_4)_2$ " Zn SO_4 extract + " " "

Reagent =C2 = " " " reagent + " " "

Extract = C3= liquid " " extract + " " "

Reagent = C4 " " " reagent + " " "

The amount of invert sugar was determined at different times by Bang's method.

Table VI

				NaF				Zn SO_4			
				Ext.	Reag.	Ext.	Reag.	Ext.	Reag.	Ext.	Reag.
1	Temp.	Hrs.	Blank	B1	B2	B3	B4	C1	C2	C3	C4
2	40°c	3	2.95	(1) 2.3	2.95	0.50	2.85	2.85	2.90	2.85	2.90
3	40°c	24	2.90	(2) 1.2	2.80	(1) 1.90	2.90	2.80	2.85	2.80	2.90
4	40°c	72	3.10	(3) 1.5	2.70	(2) 1.95	2.85	2.90	2.95	2.85	3.00
5				6.5		2.45		0.05			0.05
6		24		32.0		10.0		0.05			0.10
7		72		36.0		18.0		0.05			0.15

This table is constructed like table V. (1) 1 cc. of a dilution to 10 volumes. (2) 1 cc. of adilutionto 20 volumes, (3) 1 cc. of a dilution to 30 volumes.

From this table it is shown that much of the invertase can be adsorbed by the precipitate, but that much of it is still left in solution.

Experiment 8-5-7/2 On Invertase from liver.

In order to prove whether or not invertase is present in liver the following tests were made:

Liver - A = 2 cc. of NaF + 40 cc. of cane sugar solution.

Liver - B= " " $ZnSO_4$ + " " " " " "

Yeast - C = " " NaF + " " " " " "

Yeast - D = " " $ZnSO_4$ + " " " " " "

The yeast extracts were used merely as checks on the liver and to confirm results before stated. The amount of invert sugar was determined by Bang's method. The results are shown in the following table.

Table VII

	Hrs.	Temp.	Blank	A	B	C	D
1	4	20°c	3.2	3.2	3.2	(1) 2.2	3.2
2	24	40°c	3.4	3.3	3.3	(2) 0.4	3.3
3	4					10.	
4	24					56.	

Lines 3 and 4 show the reducing power of the invert sugar solution in terms of cc. of hydroxylamine (1) diluted to 10 volumes (2) diluted to 20 volumes.

From the above results it is seen that, there is no invertase in liver, while the value of NaF and the failure of $ZnSO_4$ as extracting agents for invertase are again confirmed.

Following are the results.

Table VIII

1	2	3	4	5
	Test	Blank	Start	24 hrs.	
1	A	11.2	10.00	4.80	5.20
2	B	11.2	10;40	6.05	3.90
3	C	11.4	10.20	6.90	3.30
4	D	11.4	10.40	8.75	1.65

Column 1 shows the test made, column 2 the blank value, column 3 the number of cc. of 0.1m $Na_2 S_2 O_3$ required to titrate to white at the beginning of the test, column 4 the number of cc of 0.1 m. $Na_2 S_2O_3$ required after 24 hours, and column 5 the difference between the number of cc, of $Na_2 S_2 O_3$ used at the beginning and the number of cc, used after 24 hours. Therefore column 5 gives the reducing power of the invert sugar solution in terms of cc. of 0.1 m $Na_2 S_2 O_3$

From the above results it is seen that after invertase had been adsorbed by $Zn_3 (PO_4)_2$, part of it at least can be redissolved and thus separated from the catalase.

Experiment 8-5-26/1 On the Separation Of Invertase From Catalase.

In order to test whether catalase remains with the $Zn_3 (PO_4)_2$ the following tests were made.

A- 10CC. of NaF extract of yeast + 1 cc.0.25 m.sol. $ZnSO_4$ x1 cc. 1.0 m.sol.KH_2PO_4

B- " " " " " " + " " " " " " " "

A- $Zn_3(PO_4)_2$ under NaF for 24 hours.

B- " " 50% acetone for 24 hours.

Experiment 8-5-22/1 On the Separation of Invertase From Catalase.

Knowing that catalase and invertase were both absorbed from a solution by absorption with $Zn_3(PO_4)_2$ and that catalase could not again be gotten into solution the following tests were made to prove whether or not invertase could be redissolved.

A-10cc. of NaF extract of yeast + 1cc. of 0.25 m sol. Zn SO_4 x 1cc. 1.0 m KH_2PO_4

B- " " " " " " + 1c " " " " " " "

C- " " " " " " + " " " " " " " "

D- " " " " " " + " " " " " " " "

The mixtures were well shaken, centrifuged, washed with distilled wate, and centrifuged again. The precipitate were placed under solvents as follows.

A- Precipitate under water for 2 hours.

B- " " " " 24 "

C- " " 50% acetone " 2 "

D- " " " " 24 "

At the end of the required time the liquids were poured off and the precipitate washed with distilled water, and added to 40 cc. of 10% cane sugar solution, and tested after 24 hours for invert sugar with 12 cc. by Schoorl's method.

After the required lenght of time both the liquid and the $Zn_3(PO_4)_2$ were tested for catalase with the following results.

A : Liquid + H_2O_2 = no change

: $Zn_3(PO_4)_2$ + H_2O_2 = large evolution of O_2

B : Liquid + H_2O_2 = no change

: $Zn_3(PO_4)_2$ + H_2O_2 = large evolution of O_2

From these results it is seen that catalase cannot be redissolved by NaF and 50% acetone.

3.

CATALASE.

Experiment 8-4-16/1 On the Extraction of Catalase. Some of the data shown in this experiment were from experiments 8-4-23/2 and 8-4-23/3.

In order to prove whether or not catalase could be extracted from yeast, and to determine which of the reagents were the most efficient, the following agents were tried.

A.-Yeast in Thymolwater - 0.5 saturated.

B.- " " Na OH -0.01 normal.

C.- " " CH_3 COOH - 0.015 normal.

D.- " " NaF - 2%.

E.- " " Zn SO_4 - 0.05 saturated i.e. about 6.7%

After the reagents had stood on the yeast for some time, some of the liquid was drawn out at varying intervals and tested for catalase as follows: About 2 or 3 cc. of the extract was placed in a test tube and an equal volume of neutralized H_2 O_2(about a 3% sol) was added. An evolution of oxygen (O_2)indicated the presence of catalase.

Following are the results showing the amount of catalase at different times.

Table IX

Ext.	4 hrs.	18 hrs.	24 hrs.	48 hrs.	66 hrs.	72 hrs.	86 hrs.
Thymol-water A	none	none	none	none	none	none	none
Na OH B	very small	small	small	rather large	rather large	rather large	small
CH_3CooH C	none	none	none	none	none	none	none
NaF D	small	small	rather large	large	very large	very large	very large
Zn SO4 E	none	none	none	none	none	none	none

From the above table it is seen that catalase can very easily be extracted from yeast by Na OH and NaF, the other reagents being ineffective or inhibiting its action.

Experiment 8-5-20/1 on Inhibition of Action of Catalase.

In order to determine whether thymolwater, CH_3COOH and Zn SO_4 inhibit the action of catalase, the following tests were made: To a liquid known to contain catalase the above reagents were added in such concentrations as to make the dilution as small as possible and preserve the reagents in the concentrations used in the previously described experiments. The results are shown below.

Liquid containing Catalase + H_2O_2 = large evol. O_2

" " " + Thymolwater + H_2O_2 = large evol. O_2.

" " " + CH_3COOH + H_2O_2 = Large evol. O_2.

" " " + Zn SO_4 + H_2O_2 = large evol. O_2.

From these results it is evident that Thymolwater, CH_3COOH and Zn SO4in the above mentioned concentrations do not inhibit the action of catalase.

It is also evident that they will not extract catalase from yeast.

Experiment 8-4-27/1 on the Adsorbtion of Catalase.

In order to determine whether catalase could be adsorbed from a liquid with precipitation, the following tests were made.

To 10 cc. of a NaF extract of yeast, 2.5 cc. of a 10% suspension of purchased dehydrated $Zn_3(PO_4)_2$ was added. After shaking thououghly the $Zn_3(PO_4)_2$ was allowed to settle. The liquid was removed and tested for catalase. The $Zn_3(PO_4)_2$ was washed twice with distilled water and tested for catalase.

Following are the results:-

Liquid + H_2O_2 = no change.

$Zn_3(PO_4)_2$ + H_2O_2 = large evolution of O_2.

This shows that all of the catalase can be adsorbed from the extract without injury to it. Thus the catalase can be removed from a large amount of liquid and held in a rather small amount of $Zn_3(PO_4)_2$.

Experiment 8-5-7/1 On extracting catalase from liver.

Knowing that liver was particularly rich in catalase and thinking that perhaps this catalase might act differently from that obtained from yeast the following tests were made.

A - Liver in NaF - 2%.

B - " " $ZnSO_4$ - 0.05 saturated.

After the liver had stood under the reagent for 24hours the liquid was tested for catalase. As would be expected catalase was present in the NaF extract, but it was present in such large amounts that it boiled out of the test tube almost at once. What was unexpected was that catalase contrary to the experiments with yeast was found in large quantities in the $ZnSO_4$extract of liver.

Experiment 8-5-14/1 on the Effect of $ZnSO_4$ in Different Concentrations upon Catalase.

To determine whether $ZnSO_4$ in different concentrations would extract catalase from liver the following tests were made:

A - Liver in $ZnSO_4$ - 0.5 saturated.

B - " " " - 0.4 " .

C - " " " - 0.3 " .

D - Liver in $Zn\ SO_4$ - 0.2 saturated.

E - " " " - 0.1 " .

F - " " " - 0.05 " .

After standing 24 hours the extracts were tested with $H_2\ O_2$ as follows:

A- Extract + $H_2\ O_2$ = slight evolution of O_2.

B- " + " = " " " O_2.

C- " + " = rather large " O_2.

D- " + " = " " " O_2.

E- " + " = quite " " O_2

F- " + " = very " " O_2.

Thus it is seen that even a 0.5 saturated solution of $Zn\ SO_4$ will extract catalase from liver to some extent, while as the concentration of $Zn\ SO_4$ decreases the amount of catalase in solution increases.

Experiment 8-5-15/1 On Adsorption Of Catalase From Liver Extract.

In order to determine whether all the catalase could be absorbed from the liver extract the following tests were made

To 10 cc. of the $Zn\ SO_4$ extract was added 0.5 cc. of a 0.2 m. solution of $Na_2\ H\ PO_4$. A white flocculent precipitate was produced, which settled to the bottom of the tube after a short time. The liquid portion was poured off and tested. The precipitate was washed thououghly and then tested. The results on the precipitate were as follows:

A - Precipitate + $H_2 O_2$ = very slight evol of O_2

B - " + " = " " " " O_2.

C - " + " = slight " " O_2.

D - " + " = " " " O_2.

E - " + " = rather large " " O_2.

F - " + " = large evol " O_2.

In no case did the liquid give any evolution of O_2 when tested with $H_2 O_2$.

Upon boiling the liquid which remained after absorption in each of the above experiments a rather heavy precipitate was obtained, thus showing the presence of proteins in solution.

The results of this experiment show clearly that the catalase can be completely absorbed from the liquid, and that the proteins areleft in solution. This gives a method of freeing catalase from these proteins.

3.3 PEROXIDASE.

Experiment 8-9-16/1 On the extraction of Peroxidase. Also, data from 8-4-23/2 and 8-4-23/3.

In order to determine whether peroxidase could be extracted from yeast by certain media the following tests were made.

A - Yeast in Thymolwater - 0.05 saturated.

B - " " Na OH - 0.01 normal.

C - " " CH_3 COOH - 0.015 normal.

D - " " NaF - 2%.

E - " " Zn SO_4 - 0.05 saturated.

Portions of the liquid were drawn off from time to time and tested as follows: 3 Cc. of a 10% solution of guaiacum in alcohol was run into a test tube. 2 Cc. of H_2 O_2 was added and thoroughly mixed. Then 1 cc. of the liquid to be tested was run into the tube in such a manner as to form a layer underneath the mixture of guaiacum and H_2 O_2. A blue color indicated the presence of peroxidase.

Table X

EXT.	4 hrs	18h.	24 H.	48 h	66 h.	72 h	96 h.	138 h.
Thymolwater A	none	none	none	none	very small	very small	none	none
NaOH B	none	none	very small	small	none	very small	very small	none
CH_3 COOH C	none	none	none	none	none	none	none	none
NaF D	very small	very small	small	rather large	small	very small	very small	very small
Zn SO_4 E	small	large	very large	very large	very large	very large	very large	very large

From the above table it is seen that peroxidase is easily extracted from yeast by NaF and Zn SO_4, while the other reagents are ineffective.

Experiment 8-4-23/1 on Inhibition of Peroxidase.

In order to determine whether Thymolwater, and CH_3 CooH would inhibit the action of peroxidase, the following tests were made:-

A solution known to contain peroxidase was diluted with thymolwater, care being taken to keep the concentration of thymol the same as in the preceding experiments. A similar solution was treated likewise with C H_3 COOH.

The following results were obtained.

Liquied containing peroxidase + Thymolwater + test = rather large amount of peroxidase.

Liquid containing peroxidase + C H_3 COOH + test = rather large amount of peroxidase.

Liquid containing peroxidase + test = rather large amount of peroxidase.

The above results show that Thymolwater, and CH_3 CooH in the usual concentrations do not inhibit the action of peroxidase. Therefore from previous data in Table No. X p. it is evident that peroxidase is not extracted from yeast by these two agents.

Experiment 8-4-27/1 On Adsorption of Peroxidase.

In order to determine whether peroxidase could be adsorbed from a liquid the following tests were made:

To 10 cc. of the extract of yeast 2.5 cc. of a 10% suspension of purchased dehydrated $Zn_3 (PO_4)_2$ was added, shaken well and centrifuged. Both the liquid and $Zn_3(PO_4)_2$ were testdd for peroxidase.

The following results were obtained.

A - NaF extract of yeast.

B - Zn SO_4 extract of yeast.

Liquid from A - no peroxidase.

" " B - no " .

Zn_3 $(PO_4$ 2)3 from A - large amount of peroxidase.

" " B - very large amount of peroxidase.

From the above data it is seen that all of the peroxidase can be removed from the solution by adsorption without inactivation of the enzyme.

Experiment 8-4-21/1 On Adsorption of Peroxidase.

In order to show more definitely that peroxidase could be easily adsorbed from a liquid the following test was made.

To 10 cc, of a Zn SO_4 extract of yeast about 1 cc. of a 0.2 m. solution of Na_2 H P $O_4$was added. The precipitate was made to settle by centrifuging and both the liquid and precipitate were tested for peroxidase. The result of this test were as follows:-

Liquid + peroxidase test = no change.

Precipitate + " " = deep blue.

This experiment shows conclusively that peroxidase is easily adsorbed from a liquid by means of the precipitation of Zn_3 (PO_4)2.

Experiment 8-5-7/1 On Extraction of Peroxidase from Liver.

In order to determine whether peroxidase could be extracted from liver the following tests were made:

A - Liver in NaF =2%.

B - " " Zn SO_4= 0.05saturated solution.

After standing 24 hours at 20° C the extract was tested for peroxidase and this was found to be present in both extracts in considerable amounts.

Experiment 8-5-14/1 On the Effect of Different Concentrations of Zn SO_4 upon Peroxidase.

In following out the perioxidase in the liver the next step was to find out if different concentrations of Zn SO_4 would extract peroxidase from liver. Accordingly the following tests were made:

A - Liver in Zn S O_4, 0.5 saturated.
B - " " " , 0.4 " .
C " " " , 0.3 " .
D " " " , 0.2 " .
E " " " , 0.1 " .
F " " " , 0.05 " .

After 24 hours the extracts were tested for peroxidase.

The results were as follows:

A - no peroxidase.
B - " " .
C - " " .
D - very small amount.
E - small amount.
F - rather large amount.

From the above results it is seen that a concentration of Zn SO_4 of more than0.2 saturated solution will not extract peroxidase from liver.

Experiment 8-5-15/1 On Adsorption of Peroxidase from Zn SO_4 extract of liver.

To 10 cc. of each of the following extracts of liver 1 cc of a 0.2 m. solution of $Na_2 HPO4$ was added. Both the precipitate and liquid were tested for peroxidase.

A - Zn SO liver extract 0.5 saturated.

B - " " " 0.4 "

C - " " " 0.3 "

D - " " " 0.2 "

E - " " " 0.1 "

F - " " " 0.05 "

The precipitate in each case gave the peroxidase test, but the liquid did also. Thus showing that some of the peroxidase be adsorbed by the amount of $Zn (PO4)_2$ used but not all of it

Upon boiling the liquid from above the precipitate a heavy precipitate was found, thus showing that the proteins had not been taken down with the precipitate.

Experiment 8-5-8/2 On Successive Extraction of Yeast Cells With Different Media.

To test the effect of successive extraction of yeast with different media the following testswere made:

A-Yeast which had been under three changes of Zn SO_4.The first was removed after 120 hours,the second after 72 additional hours,and the third after 48 additional hours.After the third extract had been removed Na F was placed on the yeast. When the Na F had stood on the yeast for 48 hours the extract was tested for peroxidase and catalase.

B-Yeast which had been under three changes of Na F was treated with Zn SO_4 as above.

Following are the results:-

A-extract ± peroxidase test = no change.
B- " ± " " = " " .
A- " ± H_2O_2 = slight evol. of O_2.
B- " ± " =-no change.

From the above results it is seen that Na F removes both peroxidase and catalase from the yeast cells while Zn SO_4 only removes peroxidase.

4. CRITICAL REVIEW AND SUMMARY OF RESULTS.

The results obtained in this work may be summarized as follows:

1.The following media have been tested for their power to extract enzymes from the yeast cell:Thymol water,sodium fluoride, sodium hydroxide,acetic acid,zinc sulphate.None of these media in the concentration used were found to inactivate any of the three ferments studied which are invertase,catalase,andperoxidase.Hence the absence of the characteristic reactions for these ferments in media which had been used for extraction indicated their inability to take the ferment into solution from the yeast cells,and not the presence of the ferments in a condition of inactivation.

2.Peroxidase is readily extracted by zinc sulphate and sodium fluoride.This enzyme can be completely removed in fully active condition from either of these two solvents by adsorbtion with zinc phosphate.The extraction of the yeast with zinc sulphate can be repeated with successive fresh quantities of the extracting medium until the no more peroxidase goes into solution i.e. until the yeast is exhausted of this ferment.

3.Catalase is not at all extracted by zinc sulphate and is readily extracted by sodium hydroxide and sodium fluoride.From either of these solvants the catalase can be completely removed in fully active condition by adsorbtion with zinc phosphate.

4.Invertase can be readily extracted by sodium fluoride as well as some of the other media,and it is very little if at all extracted from the yeast cell by zinc sulphate.The invertase can be removed from the solution in active condition by adsorbtion with zinc phosphate and from this it may be slowly redissolved by distilled water.

5.The facts developed show that the enzymes studied may be selectively separated from the yeast cells and to a large extent from each other by the following procedures:A.Removal of peroxidase by solution from the cells with zinc sulphate,thus leaving catalase and invertase still in the cells.B.After exhaustion with zinc sulphate repeatedly used,the invertase and catalase are extracted from the same cells with sodium fluoride. C.These two enzymes are both adsorbed by the same quantities of zinc phosphate formed in the sodium fluoride solvent.The catalase cannot be separated from the adsorbent but the invertase can be slowly dissolved by distilled water.